Soil nitrogenase activity of the Nylsvley Nature Reserve

P.C. Zietsman*, N. Grobbelaar[1] and N. van Rooyen [1]

National Museum, P.O. Box 266, Bloemfontein, 9300 Republic of South Africa and [1]Department of Botany, University of Pretoria, Pretoria, 0002 Republic of South Africa

Accepted 17 July 1987

A dinitrogen fixation rate of 6,3 to 8,5 kg ha^{-1} a^{-1} was recorded for the *Eragrostis pallens – Burkea africana* savanna of the Nylsvley Nature Reserve by the acetylene reduction method. The bulk of the activity occurred in the summer and autumn after good rains and appears to be largely the result of legume – *Rhizobium* symbioses. No evidence of dinitrogen fixation by free-living Cyanobacteria or symbiotic systems involving either actinomycetes or grasses was found.

Daar is deur middel van die asetileenreduksiemetode bepaal dat stikstof teen 'n tempo van 8,5 kg ha^{-1} a^{-1} in die *Eragrostis pallens – Burkea*-boomsavanna van die Nylsvleynatuurreservaat gebind word. Die oorgrote meerderheid van die stikstofbinding vind na goeie reëns in die somer en herfs plaas en is skynbaar grotendeels aan peulplant – *Rhizobium* simbiotiese-stelsels te danke. Geen aanduiding van stikstofbinding deur vrylewende Cyanobacteria of simbiotiese stelsels waarby aktinomisete of grasse betrokke is kon gevind word nie.

Keywords: Acetylene reduction, Cyanobacteria, legumes, nitrogen fixation, savanna

*To whom correspondence should be addressed

Introduction

Biological nitrogen fixation is of paramount importance in compensating for the loss of fixed nitrogen from an ecosystem. In a savanna ecosystem such as the one at the Nylsvley Nature Reserve, fixed nitrogen is probably lost mainly through fire and the marketing of herbivores although losses due to denitrification and elution during heavy rains could also be important (Bate & Gunton 1982).

In South Africa, the only estimate of the rate of biological nitrogen fixation, based on a comprehensive field study, appears to be the one undertaken during 1975/1976 by Grobbelaar & Rösch (1981) at the Nylsvley Nature Reserve. Despite the abundance of grasses, no evidence for the associative type of nitrogen fixation that Döbereiner & De-Polli (1980) claims to be operative in Brazil, could be detected at Nylsvley. Unfortunately, special provision was not made to include the possible contribution of nitrogen-fixing Cyanobacteria inhabiting the soil surface.

Because considerable variation can be expected from year to year, the work done by Grobbelaar & Rösch (1981) was repeated and extended. This paper reports on the results of the 1980/1981 survey. It deals with four experiments. In the main experiment, the nitrogenase activity of whole soil cores was monitored by means of the acetylene reduction method. However, the procedure adopted was such that the normal contribution of light-dependent Cyanobacteria growing on the soil surface would largely be eliminated. A second experiment was therefore specifically designed to ascertain whether this type of dinitrogen fixers contribute significantly to the overall nitrogenase activity of the soil. In another experiment the roots and shoots of different species of flowering plants growing in the area were separately tested for nitrogenase activity. In yet another experiment, an attempt was made to correlate the location of legumes within the study site with the localities from which active nitrogenase soil samples were obtained. This experiment also provides an impression of the relative abundance of legumes within the intensive study site.

Materials and Methods

Soil sampling sites

The study site was close to that used by Grobbelaar & Rösch (1981) and had the same type of vegetation.

A straight line 399 m long was marked out as a reference line for a hypothetical rectangular sampling grid 399 m long and 40 m wide with lines 1 m apart running parallel to the boundaries of the grid and intersecting one another at 16 000 points (excluding the points of intersection on the reference line). Each of the 16 000 points of intersection on the grid was a potential sampling point. The grid was subdivided longitudinally into 40 subsections containing 400 potential sampling points each.

Acetylene reduction

One sampling point (which had not been sampled previously) in each subsection of the grid was selected on each sampling date from a table of random numbers (Snedecor 1953). Thus 40 sampling points were selected on each sampling date.

One cylindrical soil core with a cross sectional area of 25 cm^2 was collected at each sampling point by means of a hammer-driven stainless steel soil borer. At four of the 40 sampling points (selected at random) two soil cores were collected about 50 mm apart — one of each pair was used as a control.

The soil samples were taken to a depth of 40 cm unless rock occurred at a shallower depth. Each soil sample was immediately sealed in a wide-mouthed glass container with a total capacity of about 1 100 cm^3. The screw caps of the containers were each fitted with a rubber serum-bottle stopper.

The air of the control flasks was not enriched with acetylene. Approximately one tenth of the air in the other flasks was replaced by commercial acetylene as described by Grobbelaar & Rösch (1981). After withdrawing the initial (t_0) gas samples, the soil samples were held in crates in the shade of a tree near the centre of the sampling site for 90 min before a second (t_1) gas sample was taken. A third gas sample (t_2) was collected after a further 90 min. The gas samples were stored in serum bottles as described by Grobbelaar & Rösch (1981).

Because the nitrogenase activity of many Cyanobacteria is dependant to a large extent on light (Knowles 1976; Sprent 1979), it is obvious that the above incubation procedure would largely preclude the detection of the nitrogenase activity of such surface-dwelling organisms. For that reason, undisturbed surface-soil samples were separately incubated in diffuse

light. These samples were taken from 11 randomly selected points on the sampling grid. At two of the 11 points, two samples were collected of which one of each served as a control.

A cylindrical perspex dish with a depth of 30 mm and an internal diameter of 95 mm was pressed upside down into the undisturbed soil at the sampling point until the soil surface nearly touched the bottom of the dish. After the surrounding soil had been scraped away, a steel plate was pushed horizontally through the soil against the lower (open) edge of the dish. The rim of the dish was hermetically sealed onto the steel plate by means of Prestic (Bostic Co.). Acetylene was added to the isolated soil sample through a hole in the upturned bottom of the perspex dish which had been fitted with a rubber serum-bottle stopper using the same procedure as that described for the soil core samples described above, except that the soil samples were not shaken up. The first gas sample was taken 10 min after the addition of the acetylene and at 90 and 180 min thereafter the second and third gas samples respectively were withdrawn.

The gas volume of all the sealed containers were measured by means of the method described by Grobbelaar & Meyer (1986). The ethylene concentration of the gas samples were determined by gas chromatography as described by Grobbelaar & Rösch (1981).

All the soil samples of which the ethylene concentration increased by an average of more than 260 nmol h^{-1} during the second half of the incubation period were carefully examined for legume root nodules. In addition to these 'active' soil samples, five 'inactive' soil core samples, chosen at random on each sampling date, were similarly examined for root nodules.

Nitrogenase activity of individual plant species

Individuals of the common herbaceous plant species were dug out and the roots shaken free of most of the adhering soil before the plants were subdivided into roots and shoots. For each species 100 – 175 cm^3 of shoot material and 50 – 150 cm^3 root material were incubated separately in duplicate in 250 cm^3 conical flasks fitted with rubber serum-bottle stoppers. Approximately 10% of the air in each flask was replaced with acetylene and after an initial gas sample was taken, the flasks were kept in diffuse light at ambient temperature for 5 h before another (final) gas sample was taken for gas chromatographic analysis of its ethylene concentration.

Plant surveys

A bridge point, as well as a quadrat survey was carried out. The bridge point survey (Mueller-Dombois & Ellenberg 1974) was made of the plants in the herbaceous layer. The apparatus used had 20 spikes spaced 150 mm apart. Apart from the herbs, *Dichapetalum cymosum, Ochna pulchra, Elephantorrhiza elephantina* and seedlings of all the woody species were considered to be members of the herbaceous layer during the survey. Two thousand points were examined. A strike directly on the root-bearing part of a plant was recorded as a 'full strike'. In other cases the plant whose root-bearing part was closest to the spike was recorded. The results were analyzed according to the procedure used by van Rooyen & Theron (1982).

For the quadrat survey, 1 000 quadrats (4 m × 4 m each) were superimposed on the soil sampling grid. For each quadrat only the number of individuals of each legume species was recorded. Although it is known that some species, such as

Table 1 A list of non-legume plant species occurring in the Nylsvley study area whose unwashed roots and shoots were separately tested for nitrogenase activity

Monocotyledones

Poaceae
Elionurus muticus (Spreng.) Kunth
Schizachyrium jeffreysii (Hack.) Stapf
Andropogon schirensis Hochst, ex A. Rich. var. *angustifolius* Stapf
Heteropogon contortus (L.) Beauv. ex Roem. & Schult.
Diheteropogon amplectens (Nees) Clayton
Themeda triandra Forsk.
Digitaria eriantha Steud. subsp. *pentzii* (Stent.) Kok
Brachiaria nigropedata (Munro ex Fical. & Hiern) Stapf
B. serrata (Thunb.) Stapf
Urochloa sp.
Panicum maximum Jacq.
Setaria perennis Hack.
Rhynchelytrum villosum (Parl ex Hook.) Chiov.
Cenchrus ciliarus L.
Aristida congesta Roem. & Schult. subp. *congesta*
A. mollissima Pilg. subsp. *argentea* (Schweick.) Melderis
A. stipitata Hack.
Tragus berteronianus Schult.
Perotis patens Gand.
Eragrostis lehmanniana Nees
E. pallens Gand.
Cynodon dactylon (L.) Pers.
Chloris virgata Swartz
Pogonarthria squarrosa (Licht.) Pilg.
Schmidtia pappophoroides Steud.

Cyperaceae
Cyperus margaritaceus Vahl
Fimbristylis hispidula (Vahl) Kunth

Commelinaceae
Commelina africana L.

Liliaceae
Ledebouria sp.

Dicotyledones

Illecebraceae
Pollichia campestris Ait.

Polygalaceae
Polygala sp.

Euphorbiaceae
Phyllanthus sp.

Sterculiaceae
Waltheria indica L.

Asclepiadaceae
Pentarrhinum insipidum E. Mey.

Convolvulaceae
Evolvulus alsinoides (L.) L. var. *linifolius* (L.) Bak.

Solanaceae
Solanum panduriforme E. Mey.

Acanthaceae
Justicia anagalloides T. Anders.
J. minima A. Meeuse

Rubiaceae
Oldenlandia herbacea (L.) Roxb.

Asteraceae
Schkuhria pinnata (Lam.) Cabr.

Species are arranged alphabetically within genera. The families and genera are arranged according to Dyer (1975, 1976). These species were similarly tested for nitrogenase activity by Grobbelaar & Rösch (1981)

Table 2 Frequency distribution of acetylene reduction values obtained for soil samples at Nylsvley

Sampling date	Number of soil samples that yielded C_2H_2-reduction rates (nmol h^{-1} per soil core) from:																
	−26 to −260	−1 to −25	0 to 25	26 to 260	261 to 515	516 to 770	771 to 1025	1026 to 1280	1281 to 1535	1536 to 1790	1791 to 2045	2046 to 2300	2301 to 2555	2556 to 2810	2811 to 3065	3066 to 3320	3321 to 3575
First half (90 min) of incubation period																	
1980/02/19	0	12	23	2	1	0	0	2	0	0	0	0	0	0	0	0	0
1980/03/05	0	17	20	1	0	0	1	0	1	0	0	0	0	0	0	0	0
1980/03/18	6	8	14	10	0	2	0	0	0	0	0	0	0	0	0	0	0
1980/04/01	7	7	13	12	1	0	0	0	0	0	0	0	0	0	0	0	0
1980/04/17	7	13	11	8	1	0	0	0	0	0	0	0	0	0	0	0	0
1980/04/30	6	12	19	3	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/05/15	9	19	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/05/27	9	14	14	3	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/06/03	13	10	13	4	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/07/01	10	12	14	4	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/08/08	13	10	11	6	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/09/15	16	11	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/10/27	9	8	12	11	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/11/12	2	10	11	16	1	0	0	0	0	0	0	0	0	0	0	0	0
1980/11/27	5	5	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/12/20	2	3	16	18	0	0	1	0	0	0	0	0	0	0	0	0	0
1980/12/30	2	2	14	22	0	0	0	0	0	0	0	0	0	0	0	0	0
1981/01/12	5	4	12	16	0	0	1	0	0	2	0	0	0	0	0	0	0
1981/01/26	4	6	20	9	0	0	0	1	0	0	0	0	0	0	0	0	0
1981/02/09	2	4	20	13	0	0	0	0	1	0	0	0	0	0	0	0	0
1981/02/23	4	4	15	14	0	0	0	1	0	0	0	0	1	0	0	0	1
1981/03/09	0	9	13	15	2	0	0	1	0	0	0	0	0	0	0	0	0
1981/03/23	2	6	20	10	1	0	0	0	0	1	0	0	0	0	0	0	0
1981/04/06	2	5	25	8	0	0	0	0	0	0	0	0	0	0	0	0	0
1981/04/21	4	14	18	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	139	225	387	225	7	2	3	5	2	3	0	0	1	0	0	0	1
Second half (90 min) of incubation period																	
1980/02/19	0	15	17	4	0	1	0	1	0	1	1	0	0	0	0	0	0
1980/03/05	0	5	29	1	3	0	1	1	0	0	0	0	0	0	0	0	0
1980/03/18	2	3	12	22	0	1	0	0	0	0	0	0	0	0	0	0	0
1980/04/01	4	8	12	13	2	0	1	0	0	0	0	0	0	0	0	0	0
1980/04/17	5	11	13	9	1	0	0	1	0	0	0	0	0	0	0	0	0
1980/04/30	4	4	20	10	0	1	1	0	0	0	0	0	0	0	0	0	0
1980/05/15	4	6	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/05/27	3	7	18	10	1	0	1	0	0	0	0	0	0	0	0	0	0
1980/06/03	6	9	15	8	2	0	0	0	0	0	0	0	0	0	0	0	0
1980/07/01	3	4	22	9	1	1	0	0	0	0	0	0	0	0	0	0	0
1980/08/08	3	10	20	6	1	0	0	0	0	0	0	0	0	0	0	0	0
1980/09/15	6	10	19	4	1	0	0	0	0	0	0	0	0	0	0	0	0
1980/10/27	2	10	17	9	2	0	0	0	0	0	0	0	0	0	0	0	0
1980/11/12	0	10	17	13	0	0	0	0	0	0	0	0	0	0	0	0	0
1980/11/27	3	4	18	13	1	0	1	0	0	0	0	0	0	0	0	0	0
1980/12/20	0	8	13	15	2	1	0	0	0	1	0	0	0	0	0	0	0
1980/12/30	1	11	9	14	3	0	1	1	0	0	0	0	0	0	0	0	0
1981/01/12	3	1	16	16	0	1	2	1	0	0	0	0	0	0	0	0	0
1981/01/26	1	7	17	11	2	1	0	0	1	0	0	0	0	0	0	0	0
1981/02/29	0	7	23	7	1	1	1	0	0	0	0	0	0	0	0	0	0
1981/02/23	2	8	16	10	1	1	1	1	0	0	0	0	0	0	0	0	0
1981/03/09	2	9	14	10	2	0	0	0	0	1	0	1	0	0	1	0	0
1981/03/23	3	3	25	7	1	0	0	1	0	0	0	0	0	0	0	0	0
1981/04/06	0	8	19	11	1	1	0	0	0	0	0	0	0	0	0	0	0
1981/04/21	3	8	22	7	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	60	186	438	254	28	10	10	7	1	3	1	1	0	0	1	0	0

Cassia biensis produces long rhizomes from which several aerial shoots can emerge, for convenience, each shoot that projected from the soil was in every case considered to represent a separate plant.

Results and Discussion

Acetylene reduction by various plant species

The only legumes that were encountered on the sampling grid were *Burkea africana* Hook; *Cassia biensis* (Steyaert)

Mendonca & Torre, *Elephantorrhiza elephantina* (Burch.) Skeels; *Indigofera comosa* N.E.Br.; *I. daleoides* Benth. var. *daleoides; I. filipes* Benth. ex Harv.; *I. mollicoma* N.E.Br; *Rhynchosia monophylla* Schltra.; *Tephrosia forbesii* Bak. subsp. *interior* Brummit; *T. longipes* Meisn. var. *lurida* (Sond.) J.B. Gillet; *T. lupinifolia* (Burch.) DC. and *T. semiglabra* Sond. Except for *Burkea africana* which apparently never forms root nodules (Grobbelaar & Clarke 1975; Corby 1974), all the other species were reported previously to be able to form *Rhizobium*-type root nodules (Grobbelaar *et al.* 1964; Grobbelaar & Clark 1972; Grobbelaar & Clarke 1975; Grobbelaar *et al.* 1983) and consequently they were not investigated again.

Table 1 contains a list of the 40 non-legume species which were individually tested for nitrogenase activity. All of them yielded negative results. Eleven of the species listed were tested previously for nitrogenase activity by Grobbelaar & Rösch (1981) — also with negative results.

Acetylene reduction by surface soil samples

Although eleven undisturbed surface soil samples were incubated with acetylene in diffuse sunlight for a total of 3 h on each of the following ten dates: 1980 – 02 – 17, 1980 – 03 – 12, 1980 – 03 – 27, 1980 – 04 – 11, 1980 – 04 – 24, 1980 – 11 – 20, 1980 – 11 – 26, 1980 – 12 – 31, 1980 – 01 – 18 and 1981 – 02 – 07, in no case was any acetylene reduction recorded.

The sandy nature of the soil together with the relatively low and erratic rainfall of the region was thought to make the upper soil layer a rather unsuitable habitat for dinitrogen-fixing Cyanobacteria and therefore their possible contribution to the dinitrogen-fixing activity of the soil was ignored in the study by Grobbelaar & Rösch (1981). During the present study it was, however, considered prudent to obtain experimental support for the supposition, especially because earlier studies by Saubert & Grobbelaar (1962) did attest to the abundant presence of various free-living dinitrogen-fixing Cyanobacteria as well as heterotrophic dinitrogen-fixing bacteria in the nearby swampy areas adjoining the Nyl river.

Acetylene reduction by soil cores

No ethylene production was detected in any of the control flasks.

After the results of the acetylene reduction, assays were recorded as an M.Sc. thesis (Zietsman 1982), it was discovered that the ethylene concentration of the commercial standard that was regularly used to calibrate the gas chromatograph during both the 1975/1976 and 1980/1981 surveys was considerably lower than 100%. This necessitated a correction to both sets of documented data. The results reported on below have all been corrected. Table 2 provides the frequency distribution of the acetylene reduction rates that were obtained for the first and second halves of the incubation period on each of the 25 sampling dates. Negative rates of acetylene reduction were obtained for 36,4% and 24,6% of the soil samples during the first and second halves of the incubation period respectively. Grobbelaar & Rösch (1981) ascribed the negative results mainly to the initial adsorption of ethylene onto the soil. (The detection of a negative rate of ethylene production was made possible by the fact that commercial acetylene invariably contains low concentrations of ethylene as an impurity). The negative rates of acetylene reduction that were observed during the second half of the incubation period were, however, of such a low magnitude that the mean rate of acetylene reduction for that part of the incubation period was positive for all 25 sampling dates (Table 3). By comparison, the mean rate of acetylene reduction for 7 of the 25 sampling dates was negative for the first half of the incubation period. For this reason, but also to facilitate a comparison of the present results with those obtained by Grobbelaar & Rösch (1981), the results of the second half of the incubation periods were used in the construction of curve A of Figure 1.

Table 3 Average[1] rate of acetylene reduction by whole soil samples taken from the Nylsvley Nature Reserve at different times of the year

Sampling date	Rate of C_2H_2 reduction in (nmol C_2H_4 formed) (soil core)$^{-1}$ h^{-1}	
	First 90-min incubation period	Second 90-min incubation period
1980/02/19	8,2	15,0
1980/03/05	6,3	15,5
1980/03/18	16,7	44,5
1980/04/01	30,8	44,3
1980/04/17	7,3	11,5
1980/04/30	1,4	20,7
1980/05/15	– 17,7	14,8
1980/05/27	– 12,4	15,2
1980/06/03	– 17,3	3,4
1980/07/01	– 11,6	13,1
1980/08/08	– 11,1	7,4
1980/09/15	– 20,9	2,8
1980/10/27	5,6	11,5
1980/11/12	22,8	19,5
1980/11/27	15,3	13,4
1980/12/20	51,2	80,1
1980/12/30	50,3	38,9
1981/01/12	114,5	109,2
1981/01/26	43,6	46,2
1981/02/09	59,1	39,9
1981/02/23	122,2	83,6
1981/03/09	135,3	83,7
1981/03/23	65,9	52,2
1981/04/06	11,2	13,7
1981/04/21	2,0	3,6

[1]Each figure is the mean of 40 values

By integrating the area under curve A in Figure 1, the rate of dinitrogen fixation for the experimental site was calculated to be 8,5 kg N ha^{-1} a^{-1} if it is assumed that the reduction of 3 moles of acetylene is equivalent to the reduction of one mole of dinitrogen (Grobbelaar & Rösch 1981). Conversion ratio's of 0,75 to 25 moles of acetylene per mole of dinitrogen have been used by different researchers (Hardy *et al.* 1973; Weier 1980) but several workers such as Turner & Gibson (1980) recommend that a ratio of 3:1 be used in cases such as the present one for which the ratio cannot readily be determined experimentally. The mean acetylene reduction rate for the 1980 and 1981 months of February, March and April were used in calculating the above annual rate of dinitrogen fixation.

The value of 8,5 kg N ha^{-1} a^{-1} for the Nylsvley site is slightly higher than the value of 6,3 (corrected) kg N ha^{-1} a^{-1} obtained for virtually the same site in 1975/1976 by Grobbelaar & Rösch (1981). Both values, however, are substantially higher than those obtained by several workers using the acetylene reduction method in similar field studies in the grasslands of North America. Balandreau & Dommergues (1973) obtained values that varied between 1 and 3 kg N ha^{-1} a^{-1}; Vlassak *et al.* (1973) reported 2 kg N ha^{-1} a^{-1}; Kapustka & Rice (1976) 0,7 to 3,5 kg N ha^{-1} a^{-1} and

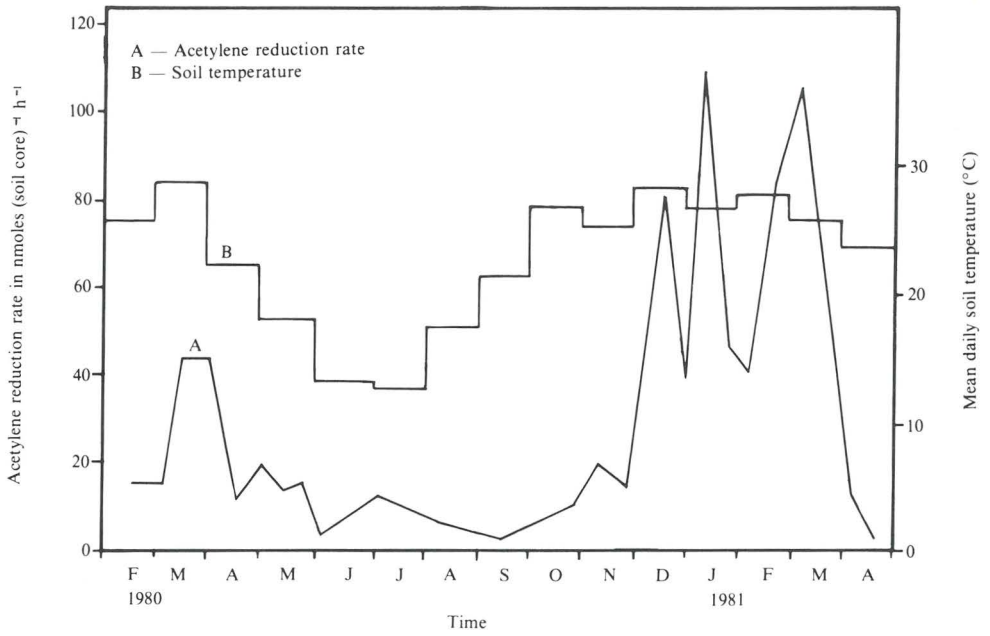

Figure 1 Mean acetylene reduction rate of soil samples (A) and mean daily soil temperature at a depth of 100 mm for the different months (B) at Nylsvley.

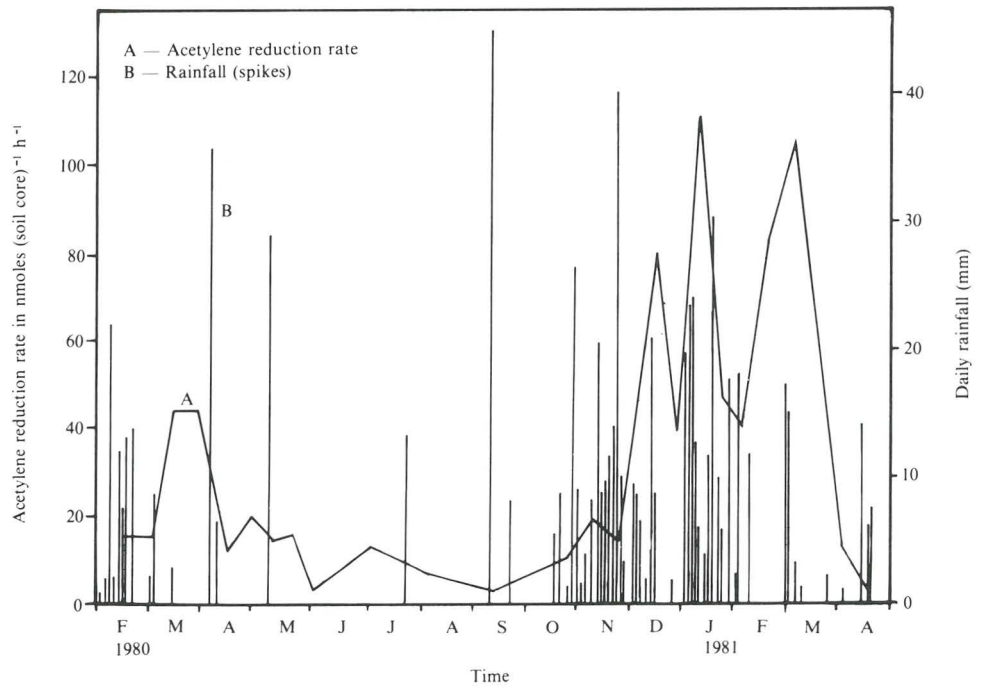

Figure 2 Mean acetylene reduction rate of soil samples (A) and daily rainfall (B) at Nylsvley.

Kapustka & Rice (1978) 3,5 kg N ha^{-1} a^{-1}. The Nylsvley rate of dinitrogen fixation is however quite similar to the 8 – 16 kg N ha^{-1} a^{-1} reported by Langkamp *et al.* (1979) and the 6,4 kg N ha^{-1} a^{-1} reported by Langkamp *et al.* (1982) for *Acacia*-rich sites in Australia. Langkamp *et al.* (1979, 1982) used ratio's of 3,9:1 and 3:1 respectively for converting their acetylene reduction rates to rates of dinitrogen fixation.

As can be seen from Figures 1 and 2, there appears to be a good correlation between the nitrogenase activity at Nylsvley and the soil temperature and rainfall pattern respectively. Most of the nitrogenase activity was found to occur during the time of the year when the upper soil has warmed up after the winter, provided good rains had fallen in the meantime. Indeed, peaks in the nitrogenase activity appear to show up about one month after good rains during the warm part of the year.

Legume-type root nodules were found in 56 (90%) of the 62 soil samples that reduced acetylene at a higher rate than 260 nmol h^{-1} during the second half of the incubation period. The six soil samples in which nodules were not found, were very wet at the time and this made the detection of detached nodules very difficult. For the 62 soil samples the mean nodule count was 1,5 per soil sample. None of the 'inactive' soil samples that were examined yielded any root nodules.

Plant surveys

From Table 4 it can be seen that *Elephantorrhiza elephantina* was the nodulating legume species with the highest density

in the herbaceous layer of the study site. Nevertheless, it had a very low percentage presence. Indeed, the nodulating legume species had such low percentage presences that it was not possible to calculate the percentage basal cover of any of them from the results of the bridge point survey.

Table 4 The percentage basal cover (A), percentage presence (B) and percentage frequency (C) of the plant species occuring in the herbaceous layer of the soil sampling site as determined by the bridge point method

Species	A	B	C
Eragrostis pallens Gand.	1,10	20,7	94
Digitaria eriantha Steud. subsp. *pentzii* (Stent) Kok	0,55	30,35	96
Justicia minima A. Meeuse	0,10	7,05	49
Aristida congesta Roem. & Schult.	0,10	4,55	47
Elionurus muticus (Spreng.) Kunth	0,10	1,20	13
Fimbristylis hispidula (Vahl) Kunth	0,05	3,50	31
Cyperus cf. *margaritaceus* Vahl	0,05	3,45	43
Rhynchelytrum villosum (Parl ex Hook.) Chior.	0,05	1,85	25
Diheteropogon amplectens (Nees) Clayton	0,05	1,55	19
Phyllanthus sp.	0,05	1,50	24
Schizachyrium jeffreysii (Hack.) Stapf	0,05	1,20	9
Dichapetalum cymosum (Hook.) Engl.	0,05	0,80	9
Ledebouria sp.	0,05	0,55	10
Commelina cf. *africana* L.	0,05	0,45	9
Bracharia serrata (Thunb.) Stapf	0,05	0,35	5
Panicum mazimum Jacq.	0,05	0,20	2
Xerophyta retinervis Bak. var. *retinervis*	0,05	0,05	1
Perotis patens Gand.		3,35	34
Aristida mollisima Pilg. subsp. *argentea* (Schweick) Meld.		2,95	32
Ochna pulchra Hook.		2,00	22
Oldenlandia herbacea (L.) Roxb.		1,60	22
Aristida stipitata Hack.		1,35	20
Pollichia campestris Ait.		1,00	13
[a]*Elephantorrhiza elephantina* (Burch.) Skeels		0,90	13
Heteropogon contortus (L.) Beauv. ex Roem. & Schult.		0,60	9
Andropogon schirensis Hochst. ex A. Rich. var. *angustifolius* Stapf.		0,40	5
Waltheria indica L.		0,40	4
Lannea edulis (Sond.) Engl.		0,35	2
Evolvulus alsinoides (L.) L. var. *linifolius* (L.) Bak.		0,30	6
[a]*Indigofera daleoides* Benth. var. *daleoides*		0,25	4
Brachiaria nigropedata (Munro ex Fical. & Hiern). Stapf		0,25	3
Justicia anagalloides T. Anders.		0,25	3
Schmidtia pappophoroides Steud.		0,25	1
Solanum panduriforme E. Mey.		0,25	3
Bidens bipinnata L.		0,20	2
[a]*Cassia biensis* (Steyaert) Mendonca & Torre		0,15	3
Pentarrhinum insipidum E. Mey.		0,15	3
[a]*Rhynchosia monophylla* Schltr.		0,15	2
[a]*Tephrosia longipes* Meisn. var. *lurida* (Sond.) J.B. Gillet		0,15	2
Themeda triandra Forssk.		0,15	1
Portulaca sp.		0,10	2
[a]*Tephrosia semiglabra* Sond.		0,10	2
Tragia rupestris Sond.		0,10	2
Hibiscus sp.		0,05	1
Gomphrena cf. *celosiodes* Mart.		0,05	1
Total	2,55		

[a]Legume species

In the case of 54 (87%) of the 62 sampling points which yielded 'active' soil samples, at least one legume-containing quadrat occurred within a radius of 2 m from the active site. In the case of 40 of the 54 cases referred to above, at least one of the legumes that could have contributed to the activity of the soil sample could have been *Elephantorrhiza elephantina*. By the above standard, *Cassia biensis* could have contributed to the activity of 36 of the active sites; *Tephrosia semiglabra* to 10; *Indigofera daleoides* to 10; *Tephrosia forbesii* to 7; *T. longipes* var. *lurida* to 3 and *Rhynchosia monophylla* to 2 whereas *Indigofera comosa, I. filipes, I. mollicoma* and *Tephrosia lupinifolia* could not have contributed because none of these species occurred in a quadrat of which one boundary was less than 2 m from an 'active' soil site.

Conclusions

Several environmental factors can influence the ratio between the rate at which a biological system will fix dinitrogen and reduce acetylene to ethylene (Bergersen 1970; Masterson & Murphy 1976; Sprent 1979; Gibson 1980; Stewart 1980; Turner & Gibson 1980; Knowles 1981; Silvester 1981). Consequently the use of the acetylene reduction assay as a method for measuring the rate at which dinitrogen is being fixed in the field yields approximate values only. This is especially so when the identity of the dinitrogen fixers is not known and the species composition of the fixers is not necessarily constant in time. Despite the above, the acetylene reduction method (Hardy *et al.* 1968) still is the most economical method of obtaining a reasonable estimate of the rate at which dinitrogen is being fixed in large fields.

There appears to be little doubt that dinitrogen fixation occurs at a relatively high rate in the *Eragrostis pallens – Burkea* tree savanna of the Nylsvley Nature Reserve with values of 6,3 to 8,5 kg N ha^{-1} a^{-1} having been recorded during 1975/76 and 1980/81 respectively. This activity which is largely restricted to the wetter part of the summer and autumn appears to be almost exclusively due to the activity of legume – *Rhizobuim* symbiotic systems (Allen & Allen 1981). Neither free-living Cyanobacteria (Gallon 1980) nor the symbiotic systems involving grasses (Döbereiner & De-Polli 1980) or actinomycetes (Akkermans & Roelofsen 1980) appear to contribute significantly to the nitrogenase activity of the region.

Acknowledgements

This work formed part of the Nylsvley Savanna Ecosystem Project and was funded by the Council for Scientific and Industrial Research of South Africa.

References

AKKERMANS, A.D.L. & ROELOFSEN, W. 1980. Symbiotic nitrogen fixation by actinomycetes in Alnus-type root nodules. In: Nitrogen fixation, eds Stewart, W.D.P. & Gallon, J.R. Academic Press, London.

ALLEN, O.N. & ALLEN, E.K. 1981. The Leguminosae. A source book of characteristics, uses and nodulation. University of Wisconsin, Wisconsin.

BALANDREAU, J. & DOMMERGUES, Y.R. 1973. Assaying nitrogenase (C_2H_2) activity in the field. *Bull. Ecol. Res. Comm.* (Stockholm) 17: 247 – 254.

BATE, G.C. & GUNTON, C. 1982. Nitrogen in the *Burkea* savanna. In: Ecology of tropical savannas, Ecological Studies 42, eds Huntley, B.J. & Walker, B.H. Springer Verlag, Berlin.

BERGERSEN, F.J. 1970. The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. *Austr. J. biol. Sci.* 23: 1015 – 1025.

CORBY, H.D.L. 1974. Systematic implications of nodulation among Rhodesian legumes. *Kirkia* 9: 301 – 329.

DÖBEREINER, J. & DE-POLLI, H. 1980. Diazotrophic rhizocoenoses. In: Nitrogen fixation, eds Stewart, W.D.P. & Gallon, J.R. Academic Press, London.

DYER, R.M. 1975. The genera of southern African flowering plants, Vol. 1. Dicotyledons. Department of Agriculture and Technical Services, Pretoria.

DYER, R.M. 1976. The genera of South African flowering plants, Vol. 2. Gymnosperms and Monocotyledons. Department of Agriculture and Technical Services, Pretoria.

GALLON, J.R. 1980. Nitrogen fixation by photoautotrophs. In: Nitrogen fixation, eds Stewart, W.D.P. & Gallon, J.R. Academic Press, London.

GIBSON, A.H. 1980. Methods for legumes in glasshouses and controlled environment cabinets. In: Methods for evaluating biological nitrogen fixation, ed. Bergersen, F.J. John Wiley, New York.

GROBBELAAR, N. & CLARKE, B. 1972. A qualitative study of the nodulating ability of legume species: List 2. *Jl S. Afr. Bot.* 38: 241 – 247.

GROBBELAAR, N. & CLARKE, B. 1975. A qualitative study of the nodulating ability of legume species: List 3. *Jl S. Afr. Bot.* 41: 29 – 36.

GROBBELAAR, N. & MEYER, J.J.M. 1986. Rapid method for determining gas volumes. *S. Afr. J. Sci.* 82: 383.

GROBBELAAR, N. & RÖSCH, M.W. 1981. Biological nitrogen fixation in a northern Transvaal savanna. *Jl S. Afr. Bot.* 47: 493 – 506.

GROBBELAAR, N., VAN BEIJMA, M.C. & SAUBERT, S. 1964. Additions to the list of nodule-bearing legume species. *S. Afr. J. Agric. Sci.* 7: 265 – 270.

GROBBELAAR, N., VAN ROOYEN, M.W. & VAN ROOYEN, N. 1983. A qualitative study of the nodulating ability of legume species: List 6. *S. Afr. J. Bot.* 2: 329 – 332.

HARDY, R.W.F., BURNS, R.C. & HOLSTEN, R.D. 1973. Applications of the acetylene – ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem.* 5: 47 – 81.

HARDY, R.W.F., HOLSTEN, R.D., JACKSON, E.K. & BURNS, R.C. 1968. The acetylene – ethylene assay for N_2-fixation: Laboratory and field evaluation. *Plant Physiol.* 43: 1185 – 1207.

KAPUSTKA, L.A. & RICE, E.L. 1976. Acetylene reduction (N_2-fixation) in soil and old field succession in central Oklahoma. *Soil Biol. Biochem.* 8: 497 – 503.

KAPUSTKA, L.A. & RICE, E.L. 1978. Symbiotic and asymbiotic N_2-fixation in a tall grass prairie. *Soil. Biol. Biochem.* 10: 553 – 554.

KNOWLES, R. 1976. Factors affecting dinitrogen fixation by bacteria in natural and agricultural systems. In: Proceedings of the 1st International symposium on nitrogen fixation, eds Newton, W.E. & Nyman, C.J. Vol. 2, Washington State University, Washington.

KNOWLES, R. 1981. The measurement of nitrogen fixation. In: Current perspectives in nitrogen fixation. eds Gibson, A.H. & Newton, W.E. Austr. Acad. Sci., Canberra City.

LANGKAMP, P.J., FARNELL, J.K. & DALLING, M.J. 1982. Nutrient cycling in a stand of *Acacia holosericea* A. Cunn. ex G. Don. I: Measurements of precipitation interception, seasonal acetylene reduction, plant growth and nitrogen requirements. *Austr. J. Bot.* 30: 87 – 106.

LANGKAMP, P.J., SWINDEN, L.B. & DALLING, M.J. 1979. Nitrogen fixation (acetylene reduction) by *Acacia pellita* on areas restored after mining at Groote Eylandt, Northern Territory. *Austr. J. Bot.* 27: 353 – 361.

MASTERSON, C.L. & MURPHY, P.M. 1976. Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field. In: Symbiotic nitrogen fixation in plants, ed. Nutman, P.S. Cambridge Univ. Press, Cambridge.

MUELLER-DOMBOIS, D. & ELLENBERG, H. 1974. Aims and methods of vegetation ecology. John Wiley, New York.

SAUBERT, S. & GROBBELAAR, N. 1962. The identification and nitrogen fixation of some free-living micro-organisms from the northern Transvaal. *S. Afr. J. Agric. Sci.* 5: 283 – 292.

SILVESTER, W.B. 1981. Acetylene reduction and the C_2H_2/N_2 ratio. In: Current perspectives in nitrogen fixation, eds Gibson, A.H. & Newton, W.E. Austr. Acad. Sci., Canberra City.

SNEDECOR, G.W. 1953. Statistical methods. Iowa State College Press, Ames.

SPRENT, J.I. 1979. The biology of nitrogen-fixing organisms. McGraw Hill, London.

STEWART, W.D.P. 1980. Systems involving blue-green algae (Cyanobacteria). In: Methods for evaluating biological nitrogen fixation, ed. Bergersen, F.J. John Wiley, New York.

TURNER, G.L. & GIBSON, A.H. 1980. Measurement of nitrogen fixation by indirect means. In: Methods for evaluating biological nitrogen fixation, ed. Bergersen F.J. John Wiley, New York.

VAN ROOYEN, N. & THERON, G.K. 1982. 'n Kwantitatiewe analise van die kruidstratum van die *Eragrostis pallens – Burkea africana* boomsavanne op die Nylsvley-natuurreservaat. *S. Afr. J. Sci.* 78: 116 – 121.

VLASSAK, K., PAUL, E.A. & HARRIS, R. 1973. Assessment of biological nitrogen fixation in grassland and associated sites. *Pl. Soil.* 38: 637 – 649.

WEIER, K.L. 1980. Nitrogenase activity associated with three tropical grasses growing in undisturbed soil cores. *Soil Biol. Biochem.* 12: 131 – 136.

ZIETSMAN, P.C. 1982. Asetileenreduserende aktiwiteit van biologiese sisteme op Nylsvley. M.Sc. tesis, Univ. van Pretoria, Pretoria.